The Stability of Palmitic Acid Monolayers

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By

Andrew Vidalis

The Ohio State University

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Defense Committee:

Dr. Heather Allen, Advisor

Dr. Doug Downey

Dr. John Shimko

Abstract

Sea spray aerosols, naturally-generated aerosols originating from the sea surface microlayer, affect the climate in a myriad of ways. To study the stability of the organic coatings on sea spray aerosols, palmitic acid monolayers served as a model system and equilibrium surface pressure values were measured on various subphase environments. Palmitic acid monolayers on lower pH subphases experienced the same equilibrium surface pressure, whereas a high pH subphase resulted in a significantly larger equilibrium surface pressure due to a desorption mechanism. The addition of 100 mM NaCl was found to improve the monolayer stability at each subphase pH level. In addition, a separate set of experiments focused on the effect of varying the $CaCl_2$ concentration of the subphase. Low $CaCl_2$ concentrations enhanced monolayer stability, while higher concentrations reduced stability. The results suggest that multiple factors of the marine and atmospheric environment are important to determining the stability of the organic films on sea spray aerosols, and hence the lifetime through which seas spray aerosols can impact the climate. Overall, information on the stability of palmitic acid monolayers can be applied to sea spray aerosols and provide some insight into the broader biological contexts of palmitic acid.

Vita

February 19, 1995……………………………Born – Akron, Ohio

August 2013 – Present……………………….Undergraduate at The Ohio State University

Publications

- Adams, E. M.; Wellen, B. A.; Thiraux, R.; Reddy, S. K.; Vidalis, A. S.; Paesani, F.; Allen, H. C., Sodium-Carboxylate Contact Ion Pair Formation Induces Stabilization of Palmitic Acid Monolayers at High pH. *Phys. Chem. Chem. Phys.* **2017**.

- Zhang, T.; Cathcart, M. G.; Vidalis, A. S.; Allen, H. C., Cation Effects on Phosphatidic Acid Monolayers at Various pH Conditions. *Chem. Phys. Lipids* **2016,** *200*, 24-31.

Field of Study

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1 Introduction

The sea surface microlayer (SSML), a gelatinous film composed of a complex mixture of ions and organic matter, is located at the ocean surface. Palmitic acid (PA) is among the various surfactants enriched in the SSML.[1] When wind acts on the ocean surface, sea spray aerosols (SSAs) are released into the atmosphere.[2-4] These aerosols have an organic coating composed of PA and other surface-active molecules.[1-2, 5] Furthermore, such naturally generated aerosols and their organic coatings play a role in affecting the local climate. The organic coatings on SSAs influence cloud formation, impact the scattering phenomena of solar radiation, and can participate in photochemical reactions.[6-7] In general, the properties of the organic coatings on aerosols influence the evaporation coefficient, and hence lifetime, of aerosols as well.[8] The goal of this project is to examine the fundamental properties of PA because it serves as a useful model for the organic film on SSAs.

The ocean environment must be considered to understand the properties of SSAs. For instance, NaCl and pH are important factors of the ocean. NaCl is known to be prevalent in sea water at a concentration of 0.468M.[9-10] In turn, Na^+ is present in SSAs.[11-12] Furthermore, the relative level of NaCl in SSAs is consequential to the climate. Aqueous NaCl aerosol particles have been shown to decrease levels of light scattering as the organic coating of the particles increased, demonstrating the potential to directly affect the climate through the scattering of solar radiation.[13] Similarly, the pH of the ocean is a critical feature of the marine environment. Generally, the bulk pH of ocean water is slightly basic at about pH 8.1.[9] Likewise, nascent SSAs are estimated to be between pH 7-9.[14] However, while it is difficult to directly measure the pH of SSAs in the atmosphere, many studies estimate that the SSAs become acidic, which could be due to equilibria with atmospheric gases such as HNO_3 and HCl.[14-16] Considering the atmospheric

implications, aerosol pH is important because acid-catalyzed heterogeneous reactions can enhance secondary organic aerosol production.[17-19] Additionally, it has been shown that the monolayer packing properties of PA are dependent on the bulk pH value and the NaCl concentration of the subphase. For instance, surface pressure – area isotherms show that PA monolayers are more expanded on subphases with high pH values and have higher collapse pressures with greater concentrations of NaCl.[20-21] Therefore, the PA model system for the organic coatings of SSAs will account for diverse pH conditions and NaCl availability in the first study presented.

In addition to NaCl and pH, $CaCl_2$ is an important component of the marine environment and it influences the properties of the organic films on SSAs. $CaCl_2$ is common in sea water at a concentration of 10.3 mM and Ca^{2+} is enriched in SSAs.[10, 22-23] In the atmosphere, salts have been shown to impact aqueous aerosols by changing their freezing temperature and heterogeneous ice nucleation ability.[24-25] At the molecular level, $CaCl_2$ affects PA monolayers. Surface pressure – area isotherms reveal that PA monolayers have higher collapse pressures relative to a water subphase and condense the monolayer at lower surface pressures while increasing alkyl chain ordering.[26] Considering the impact of $CaCl_2$, it will be incorporated at varying concentrations into PA monolayers during the second study of this work.

Generalizing beyond its usefulness to the organic films on SSAs, the stability of PA monolayers has biochemical applications as well. PA is a constituent of biological membranes.[27-29] Consequently, PA has been shown to directly impact membrane stability in ways such as raising the melting temperature of phospholipid bilayers.[30-31] PA also indirectly influences membrane stability. Protein palmitoylation promotes protein localization in cells by increasing protein-membrane interactions and PA released after nervous damage facilitates apoptosis by possibly changing the local lipid environment of receptor proteins.[32-35] Furthermore, PA changes the

properties of synthetic lung surfactants, which are utilized in the treatment of respiratory distress syndrome.[36-38] An additional medical application of PA in membranes involves conjugating cationic polymers with PA to enhance non-viral transfections for gene therapy.[39-41] Of course, biological environments can feature changes in the local concentrations of Na^+, Ca^{2+}, and H^+, so membranes containing PA should not be viewed in isolation.[42-43] Overall, PA membrane stability extends to diverse domains throughout biochemistry.

This work contains two studies that evaluate the stability of PA monolayers. For the first study, equilibrium surface pressure (ESP) experiments were conducted with PA monolayers on subphases with bulk pH values of 6.7, 8.7, and 10.7 in the presence and absence of 100 mM NaCl. These particular pH values were chosen since they represent different proportions of the protonation state of the carboxylic acid headgroup on PA, which has a surface pK_a between 8.6 and 8.8.[44] These pH values will provide information on the transition from the basic marine environment to the acidic conditions experienced by an atmospheric aerosol while also accounting for the presence of NaCl. In the second study, ESP experiments were performed with PA on subphases with various concentrations of $CaCl_2$ from 10 μM to 300 mM to assess the influence of Ca^{2+} on PA monolayers. Altogether, examining the effects of these stability factors will provide a better understanding on the stability of the organic films of SSAs and biological membranes as a whole.

2 Theory and Background

2.1 Surface Tensiometry

Surface tensiometry is the primary measurement technique utilized in this work, so a basic overview of the theory behind surface tension will be presented here. In the bulk, liquid molecules experience no net force because they are fully encompassed by neighboring molecules that equally exert forces in each direction that cancel out. In contrast, at the air-water interface, water molecules experience a net force because of the differences in the chemical potentials, number of molecules, and molecular interactions between neighboring molecules. Consequently, molecules at the surface have higher energies than in the bulk.[45] In order to achieve an energy minimum, a system utilizes surface tension to reduce the area of the interface. Accordingly, surface tension can be thought of as "the work required to change the area of the surface under reversible and isothermal conditions".[46] The work for surface tension is performed in a direction parallel to the interface.[47]

Several methods have been developed to measure surface tension, which each technique having a unique set of advantages.[48] The surface tension detection method employed in this work is the Langmuir-Wilhelmy balance method and, therefore, will be explained in detail. A Langmuir trough holds the subphase solution and has barrier(s) at the top to compress molecules at the surface of the subphase. At the surface of the subphase is a surface-active agent. For example, the surface-active agents in this work are solid crystals of PA at the surface that spontaneously form a 2D monolayer over time. A platinum or an ashless filter paper plate that is attached to a surface tensiometer acts as the Wilhelmy plate and measures the force component of the surface tension generated by the surface-active agent. The Wilhelmy plate will experience three forces (Fig. 1).
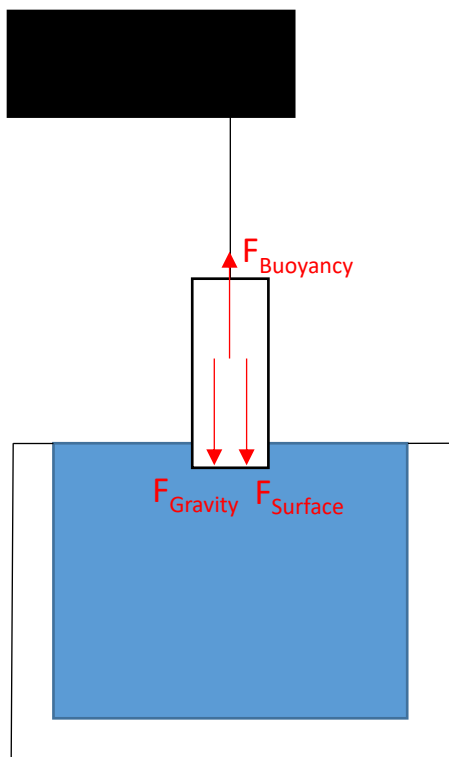
Figure 1: The Langmuir-Wilhelmy balance method calculates surface tension by having a surface tensiometer measure the three forces acting on the Wilhelmy plate.

The three forces acting on the Wilhelmy plate are the gravitational force, buoyancy, and the force associated with surface tension, each of which can be reduced to physical parameters.[45]

$$F_{Net} = F_{Surface\ Tension} + F_{Gravity} - F_{Buoyancy}$$

$$F_{Net} = 2\gamma(W + T)cos\theta + WTL\rho_{Plate}g - (WTh)\rho_{Subphase}g$$

The physical parameters include the width (W), length (L), and thickness (T) of the plate; the height of the plate submerged in the subphase (h); the densities of the plate (ρ_{Plate}) and the subphase ($\rho_{Subphase}$); the contact angle between the plate and the subphase (θ); and the surface

tension (γ). If the paper Wilhelmy plate is wetted in water, then it is assumed that the contact angle is 0°. With this assumption, cosθ = 1 and $F_{Surface\ Tension}$ is maximized. It is also assumed that the thickness of the plate is negligible. If one can measure the surface tension, then one can measure the surface pressure as well.[46]

$$\Pi = \gamma_{Subphase} - \gamma_{Monolayer}$$

The physical parameters for the surface pressure (Π) include the surface tensions of the subphase ($\gamma_{Subphase}$) and the experimental conditions with the surface-active agents at the surface of the subphase ($\gamma_{Monolayer}$). As can be seen from the previous equation, surfactants reduce the surface tension of a subphase and increase the surface pressure. Surfactants lower the surface tension of a pure subphase because the intermolecular forces of the surfactant interfere with the optimal configuration of intermolecular forces between subphase molecules.

2.2 Equilibrium Surface Pressure

In this work, surface tensiometry is used for measuring the surface pressure during ESP experiments. ESP is commonly defined as the surface pressure at which a thermodynamic equilibrium between a monolayer (2D phase) and its stable bulk phase (3D phase) occurs. [49-50] However, the vapor phase and the subphase also have equilibria with the monolayer and the stable bulk phase during ESP, although these can be ignored under most experimental conditions (Fig. 2).[51] Measurements of the ESP of a system are informative because ESP is an indicator of

monolayer stability.[52-54] A monolayer at a surface pressure above its ESP will experience relaxation via a collapse mechanism, while a monolayer below its ESP will relax by desorption.[55]
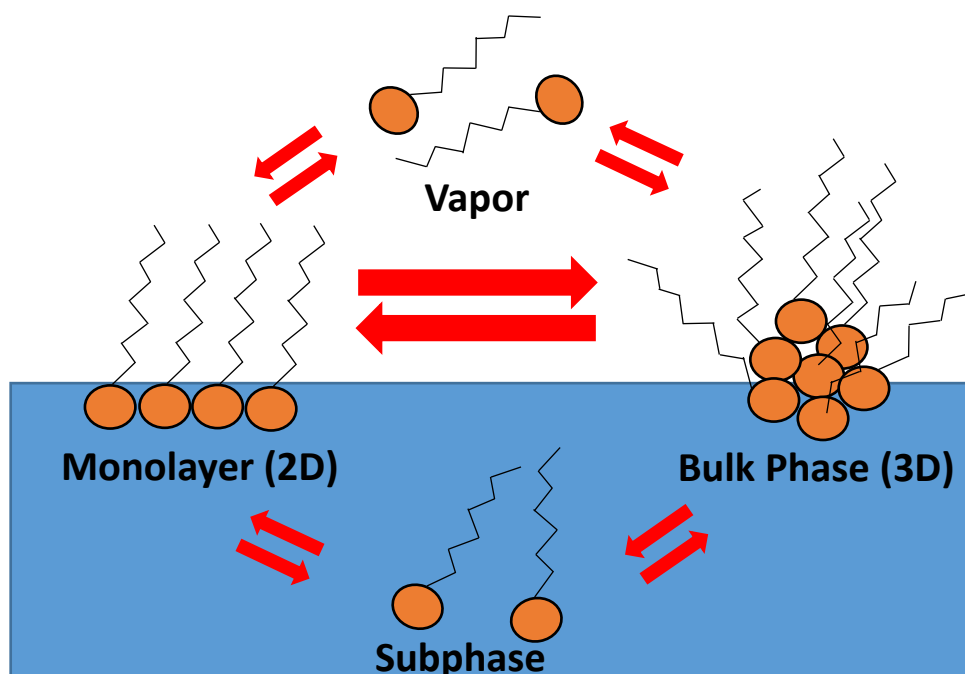

Figure 2: The various equilibria that comprise the ESP.

There are several different methods for measuring and then determining the ESP. Variability begins in the introduction of the bulk phase into the system. Commonly, crystals are sprinkled onto the surface of the subphase, but the amount and even crystal size do not follow a uniform standard between experimenters.[56-64] In contrast, the 3D bulk phase can arise from spreading a solvent containing the dissolved surfactant in quantities much larger than normal to obtain collapse structures without compression, or in normal quantities with compression to ensure collapsed films.[49, 65-67] Experiments with a liquid bulk phase are necessary for certain experiments since multiple surfactants may be investigated or a surfactant may be a liquid at the experimental temperature and pressure.[52, 63, 68]

After the decision of which bulk phase to use and what quantity, a consensus on the criteria for determining the ESP value still lacks resolution. The time for experiments can range from a couple of minutes to 36 hours.[61, 64] The ESP value is then obtained by an arbitrary parameter that could be determined by the appearance of a steady value, the inability of the surface pressure to rise by more than 0.1 mN/m in 15-30 minutes, the presence of a final surface pressure value after an extended period of time usually lasting at least 12 hours, or by fitting the curve to a manipulated first order exponential decay function in order to find the limit as time approaches infinity.[49, 52, 56-57, 64-69] Additionally, the ESP value can be verified by spreading additional bulk phase molecules, typically in the form of crystals, during the experiment and watching if the resulting change in surface pressure returns to the previous surface pressure value before the addition.[60, 62]

The ESP of a system is impacted by a multitude of factors. For instance, the experimental conditions influence the ESP of a system. Increasing temperature has been shown to result in increasing ESP values.[60, 66, 69-70] However, work with dimyristoyl phosphatidylcholine (DMPC) revealed that the ESP will remain approximately constant as the temperature increases until a critical temperature is reached. The ESP will then begin to increase as the temperatures becomes greater than the chain melting transition temperature.[71] The relative humidity (RH) during experimentation may influence the ESP as well. As the RH decreases below the saturation point of 100% RH, the rate of relaxation in constant area relaxation experiments increases. This enhanced relaxation rate was attributed to a greater rate of desorption from convection currents, which were a consequence of the nonuniform temperature gradient of a subphase experiencing evaporation.[72] This finding suggests that the equilibria involving the subphase during ESP may be dependent on the RH.

In addition to the conditions of the environment, ESP is affected by the surfactant of the system. The preparation of the stable bulk phase of the surfactant is important since the size and purity of the crystals deposited at the air-water interface alters the ESP.[60] Once prepared, differences in the structure of surfactants such as the headgroup result in unique ESP values. For example, under similar environmental conditions, the ESP for palmitic acid is 7.5 mN/m in this work and hexadecanol is reported as 39.6 mN/m, despite both molecules having a single C16 chain.[58, 60, 67] Also, the hydrocarbon chain(s) of the surfactant must be considered. The number of carbons, the degree of saturation, and geometry of the double bonds in the chain(s) have effects on ESP of varying strength.[52, 64, 67, 73] Also, changes in the chemical structure of the chain can shift the ESP value. For instance, it has been shown that different degrees of fluorination to the chain(s) of surfactants increased the ESP for a myriad of surfactants, but ω-substitutions of polar groups, including fluorine, to PA markedly decreased the ESP relative to a methyl group.[51-52, 63-64, 73-75]

Even further, ESP is dependent upon the subphase of the system. The bulk pH can have a range of effects on ESP depending on the surfactant. In a study that examined a set of bulk pH values at constant ionic strength, the ESP of dipalmitoyl phosphatidylcholine (DPPC) decreased as the bulk pH increased, while the ESP of dioleoyl phosphatidylcholine (DOPC) remained constant as the bulk pH increased.[76] Moreover, the ESP of oleic acid was larger as the bulk pH rose.[77] Additionally, dissolved molecules in the subphase may alter the ESP. Studies have shown that various inorganic monovalent salts will lead to either no change or an increase in the ESP relative to bare water.[25, 78] In comparison to water, organic molecules from ethanol to monosaccharides like glucose and fructose can decrease or increase the ESP depending on the concentration.[69]

3 Materials and Methods

3.1 Materials

Palmitic acid (>99%, Sigma Aldrich, United States) was used as received and did not undergo further purification. Solutions of pH 6.7, 8.7, or 10.7 were prepared by adding either HCl (trace metal grade, Fisher Scientific, United States) or NaOH (ACS certified, Fisher Scientific) to 18.2 MΩ•cm nanopure water (Barnstead Nanopure Filtration System, model D4741, Barnstead/Thermolyne Corporation). The pH solutions of constant ionic strength were prepared by appropriately diluting NaCl (≥99% ACS Grade, Fisher Scientific) salt solutions, which were purified by baking the NaCl salts at 650 ℃ for 10 hours, to become 100 mM solutions. The final pH of the solutions was measured (AB 15 pH meter, Fisher Scientific) to be within ± 0.1 pH units of the select values. A stock $CaCl_2$ solution was produced by dissolving the appropriate amount of $CaCl_2$•$2H_2O$ (≥99%, Fisher Scientific, ACS Grade) into the nanopure water and then undergoing a filtration process for purification.[79] The various concentrations of $CaCl_2$ required for the experiments were obtained via dilution of the stock solution. Every solution reached thermal equilibrium with the ambient surroundings for several hours before experimentation.

3.2: Methods

All experiments were performed in a Langmuir trough (Minitrough, Biolin Scientific, Finland) with an area of 145 cm^2 that was housed in a Plexiglas box to protect from dust and wind currents. To prepare the trough and the accompanying barriers, a rigorous cleaning procedure was performed that utilized rinsing with18.2 MΩ•cm nanopure water and ethanol, as well as mechanical cleaning through wiping. For the experiments, a paper Wilhelmy plate (Whatman 41, ashless grade) measured the surface pressure of the system. A sufficient surface excess of the 3D

bulk phase was achieved by depositing roughly 1 mg of PA crystals onto the bare surface, which was determined to be clean if the surface pressure was less than 0.20 mN/m upon a sweep of the barriers.[80] For the experiment, the barriers of the trough were present in an open position, but they did not compress the surface at any point. Additionally, no solvents were deposited onto the surface, so no wait time was necessary for any experiment. The experiments examining the effects of pH and/or NaCl were performed for at least 90 minutes, while experiments involving $CaCl_2$ ran for at least 3 hours. The final ESP value was determined to be the value at which the surface pressure changes by ≤0.1 mN/m in 15 minutes. Experiments were conducted at 21 ± 1 ℃, 35 ± 11% relative humidity, and ambient pressure. At least three trials were performed for each system.

4 Results and Discussion

4.1 Palmitic Acid on Different pH Subphases in the Absence of Salt

The ESP values for PA on a variety of pH subphases were determined experimentally. For a pH 6.7 subphase, PA is fully protonated and has an ESP value of 7.57 mN/m (Fig. 3A, Table 1). This value is close to reported literature values of 9.7 and 10.0 mN/m at 20 ℃ on pH 2 subphases, which should similarly have fully protonated PA molecules.[56-57] Since ESP is sensitive to the purity of the system's components, the discrepancy could be due to contaminants present in the double distilled water and/or reagent grade HCl used in these older studies. The ESP of PA on a pH 8.7 subphase is 7.59 mN/m, which is within the error of the measurement for the pH 6.7 subphase (Fig. 3B). At this subphase pH, about 50% of the PA molecules should be deprotonated at the air-water interface because the surface pK_a for PA is between 8.6-8.8.[44] Therefore, it is surprising that the ESP value of PA on a pH 8.7 subphase coincides with the value for pH 6.7. However, it is

possible that repulsive interactions between carboxylate headgroups may be balanced out by the greater availability of hydrogen bond acceptors to facilitate attractive interactions, thereby effectively canceling any changes in the forces exerted at the surface.[81-82]

While the ESP is indistinguishable for PA on pH 6.7 and 8.7 subphases, there is a large difference with a pH 10.7 subphase. The ESP of PA on a pH 10.7 subphase without any salts rises to 42.27 mN/m (Fig. 3C). In order to understand this increase, the equilibria with the vapor and subphase must be considered in conjunction with the equilibrium between the 2D monolayer and the 3D bulk phase.[51] The equilibrium with the vapor can be disregarded for PA at every pH value since the vapor pressure of PA is 1.06-7.25 x 10^{-5} Pa at 25 °C.[83] The solubility of PA in water is 7.2 x 10^{-4} g/100 g H_2O at 20.0 °C, so any solubility effects are minimized for PA on pH 6.7 and 8.7 subphases where many neutral molecules are present.[84] Yet, at pH 10.7, about 99% of PA molecules should be deprotonated according to the Henderson-Hasselbalch equation and sodium palmitate has a solubility of 0.2 g/100 g H_2O at 20 °C.[85] Consequently, the solubility of sodium palmitate is significantly greater than palmitic acid.
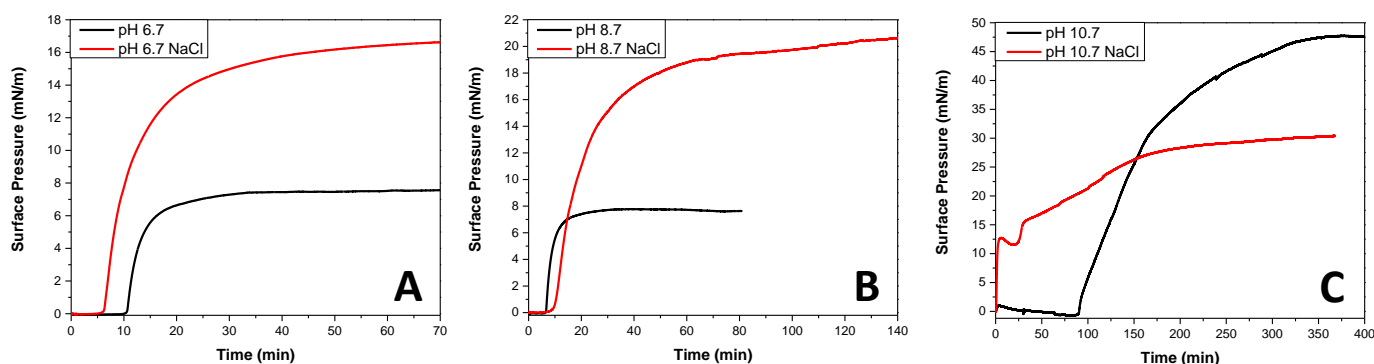

Figure 3: The ESP graphs for PA on pH 6.7 (A), 8.7 (B), and 10.7 (C) subphases in the absence of 100 mM NaCl of 100 mM NaCl.

Table 1: The ESP for each pH subphase has been calculated in the presence and absence of 100 mM NaCl.

Subphase pH	ESP without 100 mM NaCl (mN/m)	ESP with 100 mM NaCl (mN/m)
6.7	7.6 ± 0.1	17.8 ± 0.9
8.7	7.6 ± 0.2	21.1 ± 0.3
10.7	42.3 ± 5.7	31.1 ± 3.3

During desorption, the surfactant molecules first undergo dissolution into a sub-surface region that is in equilibrium with the monolayer at the surface. Furthermore, this sub-surface region experiences diffusion at a constant linear rate to the bulk (Fig. 4).[53, 86] Once the equilibrium between the monolayer and the sub-surface region occurs, there are deprotonated PA molecules in the monolayer that exert repulsive interactions with their neighboring molecules. These palmitate monolayer molecules are constrained at the surface with large electrostatic potential energy and cannot escape this energetically unfavorable condition because of the previously established equilibrium with the sub-surface region limits movement. This increase in ESP with increasing pH values due to greater electrostatic repulsive forces has already been discussed, although without mention of any desorption mechanisms.[77]
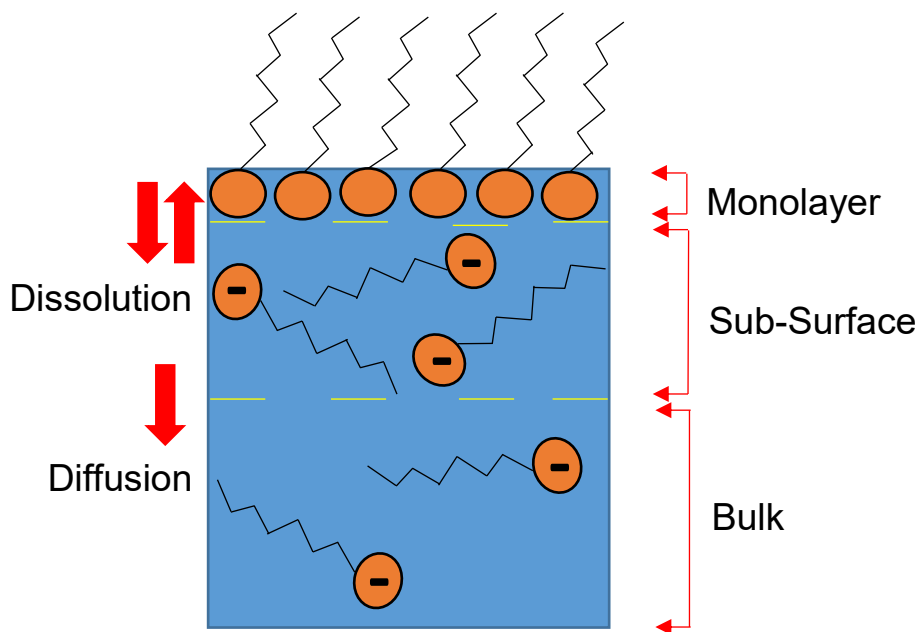
Figure 4: The desorption mechanism governing the equilibrium between the monolayer and subphase during ESP. The monolayer reaches an equilibrium with a sub-surface region during dissolution, while the sub-surface region undergoes a constant rate of diffusion into the bulk.

An additional experiment was performed to support this theory. PA crystals were spread onto the surface of a 0.4 mM sodium palmitate pH 10.7 subphase and the ESP was discovered to be 47 mN/m, which is close to the value for PA on the pH 10.7 subphase (Fig. 5). In this experiment, the subphase was at the critical micelle concentration (CMC) for palmitate, so any equilibrium with the sub-surface region would be present at the onset of the experiment.[87] As a result, the surface pressure readings were near the ESP value from the beginning. In contrast, it took about 90 minutes to see any increase in surface pressure for the experiments involving PA on the pH 10.7 subphase since it takes sufficient time to form an equilibrium between the monolayer and the sub-surface region when the subphase starts devoid of any palmitate.
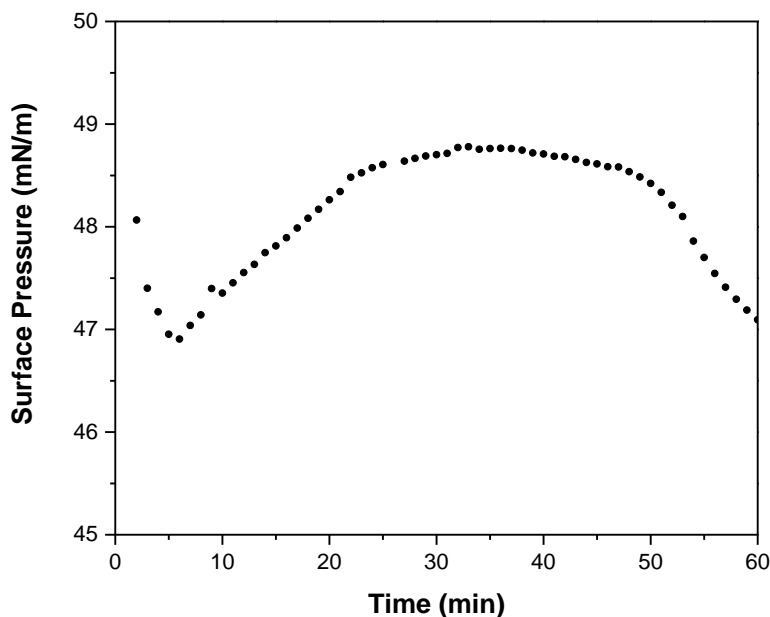
Figure 5: The ESP of PA on a 0.4 mM sodium palmitate pH 10.7 solution.

4.2 Palmitic Acid on Different pH Subphases in the Presence of Salt

Separate experiments were conducted to determine the ESP of PA on pH subphases containing 100 mM NaCl. For the pH 6.7 and 8.7 subphases containing 100 mM NaCl, the ESP values were 17.75 and 21.13 mN/m, respectively (Fig. 3A-B, Table 1). Both of these values are more than double the ESP value for PA on the respective subphase in the absence of salt, demonstrating that 100 mM NaCl markedly improved the stability of the monolayer. This rise in ESP relative to the bare pH subphases can be partly attributed to greater electrostatic repulsions in the monolayer caused by the ability of Na^+ ions to deprotonate the carboxylic headgroup of PA, which has been confirmed by vibrational sum frequency generation (VSFG) spectroscopy.[21] With greater headgroup deprotonation, and resulting electrostatic repulsion, the ESP value is higher for the pH 8.7 NaCl subphase than the pH 6.7 NaCl subphase. Furthermore, the addition of salts to a

solution drives surfactants to the interface by decreasing the free energy.[87] This phenomenon can be observed by the decrease in CMC with increasing salt concentration.[88]

PA on a pH 10.7 NaCl subphase has an ESP value of 31.08 mN/m (Fig. 3C). This measurement goes against the trend that the pH subphases with 100 mM NaCl have higher ESP values relative to their respective bare pH subphases. Other techniques are useful in explaining this apparent outlier. Infrared reflection-absorption spectroscopy (IRRAS) and molecular dynamics simulations reveal that Na^+ ions increase the surface propensity of palmitate molecules, as well as forming contact ion pairs with the COO^- headgroups of palmitate molecules that could decrease electrostatic repulsions between headgroups through charge screening.[87] Therefore, the issue is that the 2D monolayer for the pH 10.7 NaCl system is more stable, but the stability is not reflected in the ESP value since it is lower than the value for the pH 10.7 system. The pH 10.7 system has an artificially large ESP because the monolayer eventually becomes constrained by the sub-surface equilibrium to adopt a monolayer configuration with repulsive interactions. In contrast, the pH 10.7 NaCl system is not dominated by an equilibrium with a sub-surface region and has cations available to minimize electrostatic repulsions. If maintaining the integrity of the original 2D interfacial monolayer is the criterion for stability, then the pH 10.7 NaCl system is more stable than the bare pH 10.7 system. Consequently, the ESP value of a system might not be the best indicator of stability when the equilibrium with the subphase is not negligible; it should be used in conjunction with additional information.

4.3 Palmitic Acid on CaCl$_2$ Subphases of Different Concentrations[89]

A separate focus of this project is to observe how the concentration of a divalent cation modulates the ESP of a system. To accomplish this goal, ESP experiments were performed on 10 μM, 100 μM, 1 mM, 100 mM, and 300 mM CaCl$_2$ subphases, as well as with a water (~pH 5.6) subphase for reference (Fig. 6). The experiments can be analyzed in two separate groups of concentrations based on the biphasic nature of the results.

The low concentrations of CaCl$_2$ exhibit similar behavior during ESP. For concentrations up to 1 mM CaCl$_2$, the ESP values increase as the salt concentration increases (Table 2). This trend agrees with previous experiments involving subphases containing various salts with monovalent cations.[78] Additionally, a previous study with PA on subphases of different concentrations of CaCl$_2$ found a similar trend.[90] The ability of salts to decrease the CMC and increase the surface activity of surfactants may explain a portion of this finding. Overall, these findings indicate that CaCl$_2$ improves the stability of the system over the range of concentrations from 10 μM – 1 mM.

When looking at CaCl$_2$ concentrations greater than 1 mM, the systems begins to exhibit different properties. This is best evidenced by the dramatic change in shape of the curves for 100 and 300 mM compared to those at lower concentrations (Fig. 6). The graphs increase in surface pressure until a metastable state is reached and then the curves decline towards 0 mN/m as time approaches large values. This contrasts with the lower concentrations in which the surface pressure monotonically increases during these experiments to plateau into the ESP without reaching any metastable states.
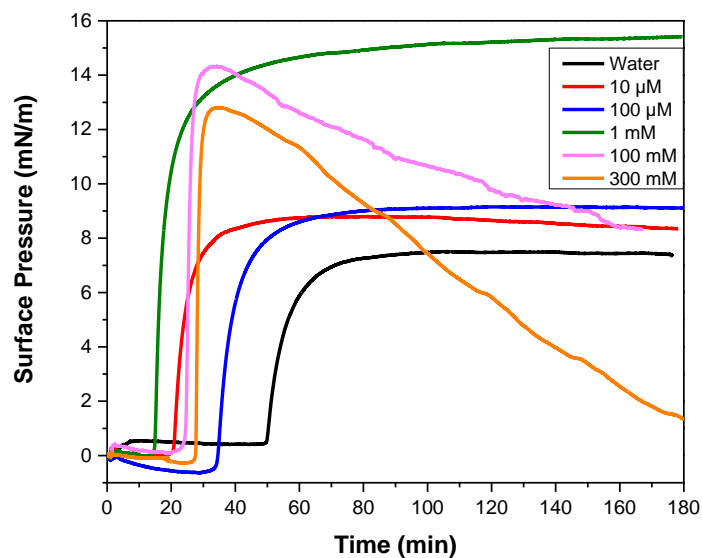
Figure 6: The ESP of PA on $CaCl_2$ subphases of varying concentration.

Table 2: The values for the ESP, metastable state surface pressure, and the rate of surface pressure decline are quantified for each $CaCl_2$ subphase. Some categories were not available (N/A) for every concentration.

$CaCl_2$ Concentration	ESP (mN/m)	Metastable State Surface Pressure (mN/m)	Rate of Surface Pressure Decline ($\frac{mN}{m \cdot min}$)
0 M	7.5 ± 0.1	N/A	N/A
10 μM	8.8 ± 0.2	N/A	N/A
100 μM	9.6 ± 0.5	N/A	N/A
1 mM	14.6 ± 0.9	N/A	N/A
100 mM	N/A	15.0 ± 0.6	-0.040 ± 0.005
300 mM	N/A	13.4 ± 0.6	-0.069 ± 0.018

The initial rise in surface pressure for the 100 and 300 mM $CaCl_2$ experiments is due to the spontaneous formation of a 2D monolayer from the 3D bulk phase of the PA crystal. This process most likely occurs until the metastable state is reached. The metastable state for 100 mM $CaCl_2$ occurs at a higher surface pressure than the metastable state for 300 mM $CaCl_2$ (Table 2). After the metastable state, there is uncertainty in the cause of the decline in surface pressure and it is currently being investigated by spectroscopic techniques. However, there is certainty that the 300 mM $CaCl_2$ subphase has a larger average decline in surface pressure per unit time (Table 2). Ultimately, the 100 and 300 mM $CaCl_2$ subphases do not promote monolayer stability like the lower concentration subphases.

5 Conclusion

Overall, multiple ESP experiments were performed in order to elucidate the stability of PA monolayers in various environments. One of the major findings was that the subphase pH can sharply fluctuate the stability of the monolayer. Lower pH values promote the greatest monolayer integrity by allowing PA molecules to stay at the interface and experience more attractive interactions. As the pH increases, the molecules initially desorb from the monolayer and then primarily undergo repulsive forces once constrained to the interface, leading to a pseudo-stable monolayer deceivingly characterized by its large ESP. Therefore, in the future, it is advised to gather other forms of data (i.e. spectroscopy, molecular dynamics simulations) to verify stability measurements obtained by ESP experiments.

The second key finding of this study is how salts impact the stability of PA monolayers. For the subphases at different pH values, 100 mM NaCl was found to create a more stable

monolayer than the subphase without the salt. While the ESP values greatly increased for the pH 6.7 and 8.7 subphases, the ESP value decreased for the pH 10.7 subphase, despite enhancing stability through Na^+:COO^- contact ion pair formation. Furthermore, there was a separate investigation into the effect of salt concentration. $CaCl_2$ was determined to enhance monolayer stability with increasing concentration up to 1 mM. After that concentration, the monolayer stability was compromised. This biphasic behavior for a salt with a divalent cation is significant because salts with monovalent cations have been shown to monotonically increase, or have no effect, on ESP with increasing activity.[78] All factors considered, salts generally increase the stability of a monolayer, but may only do so until a critical concentration.

These experimental conclusions provide useful insights into the properties of SSAs. It is possible that nascent SSAs generated at the basic oceanic pH may contain micellar structures. However, subsequent acidification in the atmosphere should lead to a more stable organic film. Additionally, the local salt environment of the marine water producing the SSAs is important in determining the stability of the organic film surrounding the aqueous core. The identity and concentration of each salt will modify the interactions between the monolayer molecules, so each marine environment will be different depending on the time and location. The confluence of all these factors is necessary to understand how the organic film will behave in the atmosphere and influence the diverse climate implications of SSAs. Further investigations into the composition of the SSML of various ocean environments across the world will allow for tailored research on the effects of assorted salts and pH conditions on PA. With this information, the effect of SSAs on the climate can be quantified more accurately. Expanding to the broader biochemistry applications, PA is strongly influenced by common biological variables. Therefore, additional research on stability for membranes containing PA should account for Na^+ ions, Ca^{2+} ions, and pH levels.

6 References

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